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October 24-26, 1989

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U. S. Department of Energy
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November 3-4, 1989

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11/1/89

U. S. Department of Energy
Human Genome Program
Contractor/Grantee Workshop
Santa Fe, New Mexico
November 3-4, 1989

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EXHIBIT J

EXHIBIT REDACTED IN ITS ENTIRETY

EXHIBIT K

EXHIBIT REDACTED IN ITS ENTIRETY

EXHIBIT L

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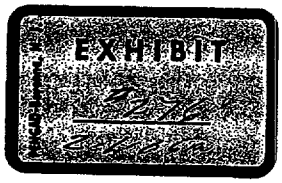
December 7, 1989

Dear Rasha,

I am sending you this fax to discuss some things that were not mentioned in my recent letter (sent via airmail to you and to Rada a few days ago). First, to repeat some things that were in the letter, Stefan and I have been working on a business plan for Genomic Solutions, Inc. (GSI) which he will send to a potential investor within the next few days. Stefan has identified several people who are interested in seeing a business plan, and he is quite confident that we will be able to get seed funding of about $500,000 in Jan. or Feb. to organize the business and start the research projects. Also, I have obtained some of the Teflon support for making support-linked oligos, and will start working with it very soon.

Stefan thinks that it might be worthwhile to look into setting up formal collaborations with other groups working on SBH, such as the Moscow group and/or the Oak Ridge group. We won't pursue any of these possibilities without your approval and participation. The main point is that since these other groups are obviously working on projects related to SBH, and some of these other approaches may fall outside of your patents and may succeed, it may be best to establish communications and coordinate the projects early on, to minimize conflicts, prevent duplication of effort, and work toward mutual commercial benefits.

The least urgent situation is the Soviet group. They may be able to offer manpower and nucleic acid chemistry expertise that



could facilitate some aspects of the DNA chip construction. If so, we might start thinking about ways of collaborating with them. What do you think about this?

The more urgent case is the Oak Ridge group -- urgent only because they have asked me to write a supporting letter of collaboration for a grant application which is due next week, and I won't make any commitment to assist in their project unless you recommend it. They specifically stated that the project would be a collaboration with you, too, since they agree that the use of beads for SBH is your "territory." As Bruce wrote in his letter to you, they propose to make CPG tagged by combinations of stable isotopes and derivatized in a way which makes a CPG-DNA linkage that is stable to the deprotection condition. They would like to supply these beads to me for use in synthesizing large numbers of oligonucleotides. The beads would then be used in a different kind of SBH implementation than yours. The grant (Mike Ramsey at Oak Ridge is Principal Investigator) proposes to use the beads for hybridization to fluorescent-tagged genomic fragments, then isolate the fluorescent beads by single droplet RIS and fluorescence sorting, then identify the sequences by their "isotopic signature." Are you agreeable to this collaboration?

Can you phone or fax me with your thoughts about this collaboration? My personal view is that the beads that they would be supplying would also be helpful in your currently planned SBH projects, and that additional mutually beneficial collaborations could be developed if our groups worked together. A copy of Bruce's letter to me is attached. Two more things: (1) Can you fax me a copy of your resume (for the business plan)? (2) If necessary to get the startup funding, would you afford to visit here in early or mid January?

Best wishes,

Ken

Kenneth L. Beattie
tel (713) 798-5762
fax (713) 798-5555

12/7/89

Biology Division
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P.O.Box 2009
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Dr. Kenneth Beattie
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Baylor College of Medicine
One BAylor Plaza
Houston, TX 77030 Fax 713 798 5555

Dear Ken,

We are preparing two grant proposals that will deal with different aspects of the SBH procedure. Bob Foote is describing the 'chip' and Mike Ramsey is describing the CPG bead labeled with multiple stable isotopes. You did not meet Ramsey in October but he came to Crkvenjakov's seminar. Mike is knowledgable in the area of detecting single fluorescent molecules and wants to develop this into a practical assay in some areas involving DNA. As I described to you on the phone we propose to label beads with three stable isotopes; using five isotopes each of five elements there are over 10,000 combinations. Each bead would have a unique oligonucleotide attached and when hybridized to a fluorescently labeled DNA the bead would have fluoresence and be detected in a microdroplet that could contain only one bead at a time. The fluorescent bead would be divereted, in a sorter like the cell sorter, and the non-fluorescent beads discarded. The fluorescent bead would then be characterized for the combination of stable isotopes.

We propose an experiemt to label the beads with ten combinations of stable isotopes of gadolinium. We are proposing that you would synthesize appropriate oligos on these beads and we would test them for proper hybridization.

If you are agreeable to this kind of collaboration would you prepare a letter to J. Michael Ramsey, Analytical Chemistry Division, ORNL, P.O. Box 2008, Oak Ridge, TN 37831. It is very near the deadline and we would like to receive the letter by Dec 11.

I wrote to Belgrade to tell them about this but have not received a reply yet. I told them we would consider any "bead" project a collaborative one with them since we agreed to have beads be their territory.

With two approaces to SBH here and others in Belgrade it seems that something should work out. Some careful work on the specificity of hybridization is one of our top priorities. Please let me know as soon as you can if you can agree to this arrangement.

Sincerely yours,

Bruce

K. Bruce Jacobson
Biology Dvision

TOTAL P.01

TELEFAX COMMUNICATION

DATE: Dec. 7, 1989
TIME: 4:15 p.m.

TO: Dr. Radomir Crkvenjakov
Genetic Engineering Center

FROM: Dr. Ken Beattie
Baylor College of Medicine

NUMBER OF PAGES, INCLUDING COVER: 1

MESSAGE:

Rasha,

Things are exciting here. I hope you can send me a fax message today (Dec. 8)

Ken

EXHIBIT M

SEQUENCING BY HYBRIDIZATION (SBH) R&D at the CENTER FOR GENETIC ENGINEERING in BELGRADE, YUGOSLAVIA: The RADOMIR CRKVENJAKOV LABORATORY in 1989
Trip Report by Marvin Stodolsky

Summary The purpose of this trip was to review progress of SBH R&D in the laboratory of Assoc. Prof. R. Crkvenyakov. His laboratory is developing a novel scheme for genome Sequencing By Hybridization (Dramnac et al, 1989, Genomics 4, 114-128), the interrogation of representative clonal DNAs with short DNA strands (oligonucleotides). It was reviewed by a 1988 DOE Special Review Panel for the Human Genome Initiative and is now jointly funded by DOE and the Center for Genetic Engineering in Belgrade, Yugoslavia. The Center is a non-profit institution sharing staff and graduate training responsibilities with the University of Belgrade.

The project further benefits from a 1980 US-Yugoslavia Treaty for Scientific and Technological Cooperation. Projects considered meritorious by a US-Yugoslav Joint Board have a special financial privilege. Yugoslav dinars can be exchanged for US dollars to facilitate the import of foreign research materials. Also funds are provided (through the US Dept. of State and its Embassy) for Yugoslav scientists to visit the US colleagues and vice versa. My 1988 and this May 28-June 9, 1989 site visit to the Center was made under these auspices. In the DOE Office of Health and Environmental Research, I am technical representative for several of the DNA sequencing technology projects within the Human Genome Initiative.

From the onset of the SBH project it was evident that a full implementation of human genome sequencing by SBH would require cooperation with US scientists/technologists, particular as development moved from biological/biochemical proof of concept to instrument/automation intensive phases. Thus my responsibility for this trip was to evaluate progress, facilitate the program as possible and bring back recommendations for further actions.

Progress in 1988-89 has been considerable. Most outstanding are improvements in DNA interrogation procedures and a scheme for process miniaturization. The SBH team are now seeking collaborators to develop process constituents for which the Center is not adequately equipped.

Itiniary May 27-Departure from Wash. DC, arriving in Belgrade May 28.
Monday, May 29-June 9, in Belgrade and surrounds.
Saturday June 10-Thursday June 21 in Istanbul, Turkey as guest of Prof. Engin Bermek and Dr. Cafer Yildiran. This portion of my trip was taken as yearly vacation. I taught in Turkey from 1980-1982 and retain numerous collegial friendships there. Dr. Bermek is director of the Molecular Biology Section of TUBETAK (Turkey's NSF equivalent with both funding responsibilities and its own laboratories) and administrator of a NATO R&D Award for a multifaceted biotechnology program.
Thursday, June 22-return to Wash. DC.

Meetings/Personalities The first week was largely spent absorbing details of the years effort. My first two day's overlapped a visit by Daniel Jacobson (to be at Johns Hopkins U. this fall). He is developing some very interesting SBH instrumentation ideas with Crkvenyakov. The concepts are however proprietary

and cannot here be described. Ivan Labat (Ph. D. candidate) is writing further software for SBH analysis. Belgrade's almost daily power failures have been causing loss of enroute computations; to minimize these we designed a simple power backup to preserve data in computer RAM, which Labat will implement.

The day of June 7 was largely spent at the US Embassy. The science attache Robert Day, his assistant Anna Druzetic and other staff elicited from Crkvenyakov and me a description of the Human Genome Initiative, the DOE/NIH roles and the niche of SBH. I promised to put them on the Human Genome mailing list and left them copies of the 1988 genome program pamphlet and the current DOE genome news letter. The Embassy's video communications facilities were demonstrated. Crkvenyakov subsequently utilized them, as one of six participants worldwide in a June 21, USIA sponsored WorldNet forum on the Human Genome Initiative. In the afternoon scientific attache Day, Crkvenyakov, V.Z. Ascher and I met with Ambassador Zimmerman and responded to questions about the Genome Initiative and SBH. This was the Ambassador's first personal contact with Crkvenyakov, though he had cited the SBH project and the WorldNet broadcast during the May meeting of the US-Yugoslav Joint Board. Vivienne Z. Ascher (my wife) had joined me for the second week in Belgrade. During 1980-82, she had served in the US Embassy in capacities of scientific, banking and finance officer. Her knowledge of Serbo-croatian, Yugoslav culture and protocol facilitated many contacts.

On June 8, I responded to a request for a group meeting from Center staff, for my assessment of the SBH project. My primary points were that SBH development is indeed progressing well, and that they should begin to consider the new problems/opportunities the Center would encounter if it does become a genome sequencing method of choice. Movement from current proof of concept efforts to effective genome sequencing would require much supportive infrastructure if the Center/Yugoslavia wished to obtain optimal benefits. At a minimum, they could serve as a training center for SBH technology. Maximal benefits could accrue from offering a commercial genome sequencing service, but that would take long range planning, economic modeling and much cooperation from their government.

Indeed, much of the second week was spent considering alternative development plans with Crkvenyakov. They had two common themes. The SBH software will be made publicly available, so that the efficacy of the sequencing assembly algorithms can be independently verified and further improved. Secondly, a full technical demonstration of the multidisciplinary system can most easily be accomplished in the US. A finalized system could be reproduced in Yugoslavia if they can develop the supporting infrastructure. An alternative would be to establish a SBH facility in Trieste at the International Centre for Genetic Engineering and Biotechnology (for developing countries), with which the Belgrade Center has close ties.

In all my contacts, I emphasized that I was merely a scientific advisor to the DOE, and my analyses/suggestions were in no way binding or policies of the DOE Office of Health and Environmental Research.

The Center for Genetic Engineering. Some current circumstances concerning the Center bear mention. Ground has now been broken for their dedicated research building, with structural completion scheduled for Fall 1989. Eventually the School of Pharmacy of the U. of Belgrade will be situated beside it. There are

currently three Center projects benefiting from the US-Yugoslav treaty: the SBH co-funded by the Center and DOE; Prof. D. Savic's (NIH) project on a strong mutator action of the polA1 allele in Escherichia coli; and Prof. V. Glisin's (NIH) Belgrade rat model for thalessemia. The international recognition of these projects gives the Center high visibility, and leverage to illustrate the values of good R&D to capable Yugoslav students. The major change in the Crkvenjakov group has been the departure of SBH theoretician Radoje Drmanac. He has begun a postdoctoral in the laboratory of Hans Lehrach at the ICRF in London, but remains a continuing force in SBH development.

SBH R&D during 1988-89. The Lehrach and Crkvenjakov laboratories utilize many common technologies and are constantly exchanging protocol improvements. The idea for fingerprinting a clone by its content of a number of oligomer sequences originated with Lehrach. Residence/absence of a particular sequence is determined by hybridizing a corresponding DNA oligomer against the DNAs of a clone. In the SBH implementations, clonal DNAs are excreted from intact bacteria in the form of M13 virions with up to 17 kb single stranded DNAs, of which up to 10 kb is foreign insert. When as little as 0.1 ul of virion supernatant (about 50 million virions or 0.1 ng of DNA) is spotted on a blot membrane, adequate signal is obtained in subsequent oligomer interrogations. Oligomer fingerprinting applications have not been as successful on cosmid or bacteriophage lambda dot blots. With their much larger 50 kb chromosomes, the number of chromosomes per ug DNA deposited in a dot is much lower and bacterial DNA debris are present, with attendant severe signal/noise problems.

The core goal of SBH is to obtain the sequence of a chromosome or genome as an assemblage of its constituent short oligomer sequences. Through computer modeling optimal system configurations are chosen. In the published scheme, a family of 100,000 oligomer probes is necessary, comprised of a family of some 60,000 eight-mers plus strategic longer oligomers. Complementary "ordering" and "basic" libraries are produced with 0.5 and 10 kb inserts respectively. This configuration would be optimal for 1-5 megabase range chromosomes, such as bacterial or YAC chromosomes. A library of about 10 million clones would be necessary for SBH sequencing of the total human genome. Early oligomer residence data gathered orders the clones. Eventually the entire sequence of the source chromosome (or genome) can be assembled as its set of overlapping oligomers. Enroute ambiguities in the oligomer overlaps are resolved by the clone order information. This is the core of SBH theory.

Several developments of the past year deserve summary. There has been further improvement of computer algorithms. They are needed to optimize specifications of the basic and ordering libraries, in terms of the redundancy of chromosome/genome coverage, for a particular SBH implementation. The longer the oligomers utilized, the larger the clonal inserts and the smaller the libraries can be. But the number of oligomer hybridizations and associated costs also rapidly increase with oligomer size. Thus there are trade offs to be made in the economics of process constituents. Several computer scientists have become interested in these problems. In the US, Ross Overbeek (ANL) and Karl M. Sirotkin (LANL) have/are cooperating. In the USSR, a completely independent development of SBH mathematics appears to have been initiated (see attached references).

In Belgrade, the software was initially written in the BASIC programming language. At the Human Genome Workshop at Cold Spring Harbor this April, an

informal group including K. Sirotkin, E. Branscom and D. Waterman (of the Lander-Waterman ordering models) advised Crkvenjakov on future preferred software implementions. They recommended that the algorithms be translated into the more popular C language. Cooperation with and improvements by other scientists could thus be much more easily achieved. Crkvenjakov accordingly purchased Turbo C compiler software before departing, and programmer Labat is now enthusiastically doing the BASIC to C conversion.

The group also considered routes to economically improving the Center's computation capacities. Details on the Center's hardware were FAXed to me and forwarded to LANL. Before leaving on this trip LANL specialists were able to advise me on suitable electronic boards for increasing RAM to 4 megabytes on the Center's PC/AT compatible. This would permit much faster modeling and optimization at the Center. Examination of this computer confirmed that there are no compatibility problems. Also Belgrade now has a BITNET nexus, so electronic mail transmission to the Center is forthcoming.

Perfection of an essential SBH technique was reported by Crkvenjakov at Cold Spring Harbor. It is the capacity to unequivocally discriminate between a perfectly paired oligomer/target DNA complex and any corresponding oligomer/target with even one mis-paired residue. The theoretical analysis depicted a two step process. The first is a loading of targets with enough labeled oligomer to subsequently give an adequate signal. This loading does not have to be highly selective. The second step is a thermal dissolution of complexes, which would eventually release perfectly paired as well as mis-paired oligomers. But the dissolution rate is higher for the mis-paired oligomers, and the differential between the rates is highest at low dissolution temperatures. Thus (contrary to some earlier intuitions) the best discrimination and signal are achieved by: an initial low specificity oligomer loading; a slow dissolution of complexes during which the perfectly paired complexes best survive; and a stabilization of surviving complexes, after which the readout signal of the bound oligomer is acquired. In the corroborating experiment, effective discrimination was cleanly demonstrated for complexes differing by a mis-pairing in a terminal residue of the oligomer. This represents the most difficult discrimination situation. Thus a critical milestone has been passed.

A year ago it seemed that 11-mers were the shortest oligos which could be utilized in clone interrogations. But SBH interrogations with oligomers shorter than eight subunits have now been achieved, which permit substantial savings in synthesis costs. Also utilizing shorter oligos permits the utilization of lower temperatures during target DNA interrogations. One consequence is that more labile process constituents can be expected to have longer lifetimes, during the clone interrogation cycles. While demonstrations have as yet not been pursued, 1000 cycle longevity of dot blots or the DPs described below would not be surprising.

Some other presentations at Cold Spring Harbor have relevance to SBH. Firstly, K. Beattie (Baylor U.) reported on a system for more economical synthesis of Oligomer Banks relevant to a full SBH implementation. The cost determining factors on the number of oligos and the minimum amounts for a practical synthesis. For the current minimum practical quantity of 0.2 micromoles and 100,000 oligos, the cost is between $0.5-1.0 million. But the 0.2 micromoles will further suffice for analysis of a million genomes of human size by SBH. Also the Oligo Bank would be a source for producing PCR primers

very economically by ligation. Thus the initial Bank cost would be greatly amortized by continuing uses.

Secondly, there has been an increasing effective development of non-radioactive reporting labels for DNAs and oligomers. The importance is that these labels report with light. Consequently, source sizes down to their wavelengths (less than a micron) can be spatially resolved. In contrast, the prior use of radioactive labels required minimal source separations of about 0.5 millimeter, because of the pathlength of the reporting electron emissions. In addition photon signals are now acquired by efficient quantitating CCD devices. This provides for automated recording of and electronic processing of digitized data. These benefits were evident in several analyses of metaphase chromosomes by fluoresence microscopy.

The capacity to resolve compact sources motivates a new direction in the practical implementation of SBH, described in a manuscript "Prospects for Miniaturized, Simplified and Frugal Human Genome Project" by Dramnac and Crkvenyakov. Heretofore clonal DNAs to be analyzed have been immobilized on blot membranes. With a clone spot per square millimeter, 10 million clones needed for human SBH and an estimated blot longevity of 100-1000 (of 100,000) hybridization cycles, some 1000-10,000 square meters of blot membranes would be necessary. Costs of reagents scale with the surface area (and roughly the same economics apply to conventional sequencing). To achieve economies, D&C are advocating the use of small Discrete Particles (DPs) as the holders of clonal DNAs in SBH. The defining characteristic of a DP is that each DP is identifiable within a mixture of thousands, in addition to its suitability as a stable solid/matrix for SBH interrogations. A stratagem for distinguishing DPs is currently being patented. After DPs irreversible bind their recombinant DNAs in wells of microtitre trays, they can be pooled without information loss and fixed in a layer upon a suitable carrier.

A square centimeter of microscope slide could carry a million DPs with 10 micron diameters. For a fail safe 10 fold redundancy in representation of the DPs, a 100,000 distinct DPs would be carried on a slide. Thus the 10 million M13 clones required for a complete human genome sequencing could be carried on 100 slides. In preliminary interrogations of the slides, the location and identity of each DP would be established. Then these slides would be run through automated cycles of:

1. The fast stripping of oligomers bound during the prior cycle;

2. The fast binding of multiple oligos with distinguishable labels;
3. A "slow" 45 minute elimination of mis-paired oligos;
4. Oligomer label excitation/readout on the microscope stage with automated database entry.

Assuming 10 distinguishable fluor labels for oligos, 10,000 1-4 cycles would extract the sequence data. Assuming that Step 4 readout needn't be rate limiting, the 1-4 step oligo cycle time is about an hour and 420 days would be required for data readout from slides. With hybridizations of replicate slides with different oligomers in parallel (or time staggered sequences), this time would be correspondingly reduced. Assuming a 1000-100 replicate slides required by a 100-1000 cycle DP longevity, data acquisition from slides would take 1-5 days if mechanics and optical readout are not limiting.

The complete human genome sequencing would have the following components:

A. Preparation of 10 million recombinant M13 virion supernatants;
B. DNA binding to DP and DP mount preparation;
C. The above described 1-4 cycle DP-oligo readout;
D. Bridging centromeric/telomeric repeat regions with YACs.
E. Eliminating any final ambiguities by standard sequencing of PCR amplified segments; needed primer sequences would be known from the SBH data.

In summary, the process miniaturization and short cycle times make SBH on DPs a very interesting candidate for total genome sequencing, and the follow on sequencing of subsequent genomes of biotechnological/agricultural interest.

The Future The Belgrade team with R. Drmanac are now seeking collaborators to aid implementation of Frugal Genome Sequencing. On the theoretical side, more computational modeling and optimization is desirable before large investments in oligomers and libraries are begun. On the physico-chemical side, optimization of DP properties/preparation are the next necessary step. Modes of 2D immobilization of pooled DPs on slides require test and verification. Proof of concept DP interrogations can utilize already sequenced M13 recombinant DNAs or a library representing a genome of moderate size. Kb

In the latter application, an ordered library (of clones with 10 ~~megabase~~ inserts) would be an enroute product, serving for validification tests before committment to a full 100,000 Oligo Bank construction. Readout from the microscope stage can begin with current fluorescence instrumentation. But total process time can be greatly decreased by automated readout and data recording.

The SBH team have requested that I send my Trip Report to potential collaborators. Collaboration could include a visit by Dramnac to a US multidisciplinary lab, after completion of his current postdoctoral with Lehrach. I can provide some futher information or contacts can be made at the below addresses.

Marvin Stodolsky, on detail with the
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EXHIBIT N

EXHIBIT REDACTED IN ITS ENTIRETY

EXHIBIT O

EXHIBIT REDACTED IN ITS ENTIRETY

EXHIBIT P

EXHIBIT REDACTED IN ITS ENTIRETY